Application of MS-based footprinting in drug discovery and development

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STRUCTURAL MASS SPECTROMETRY
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Outline

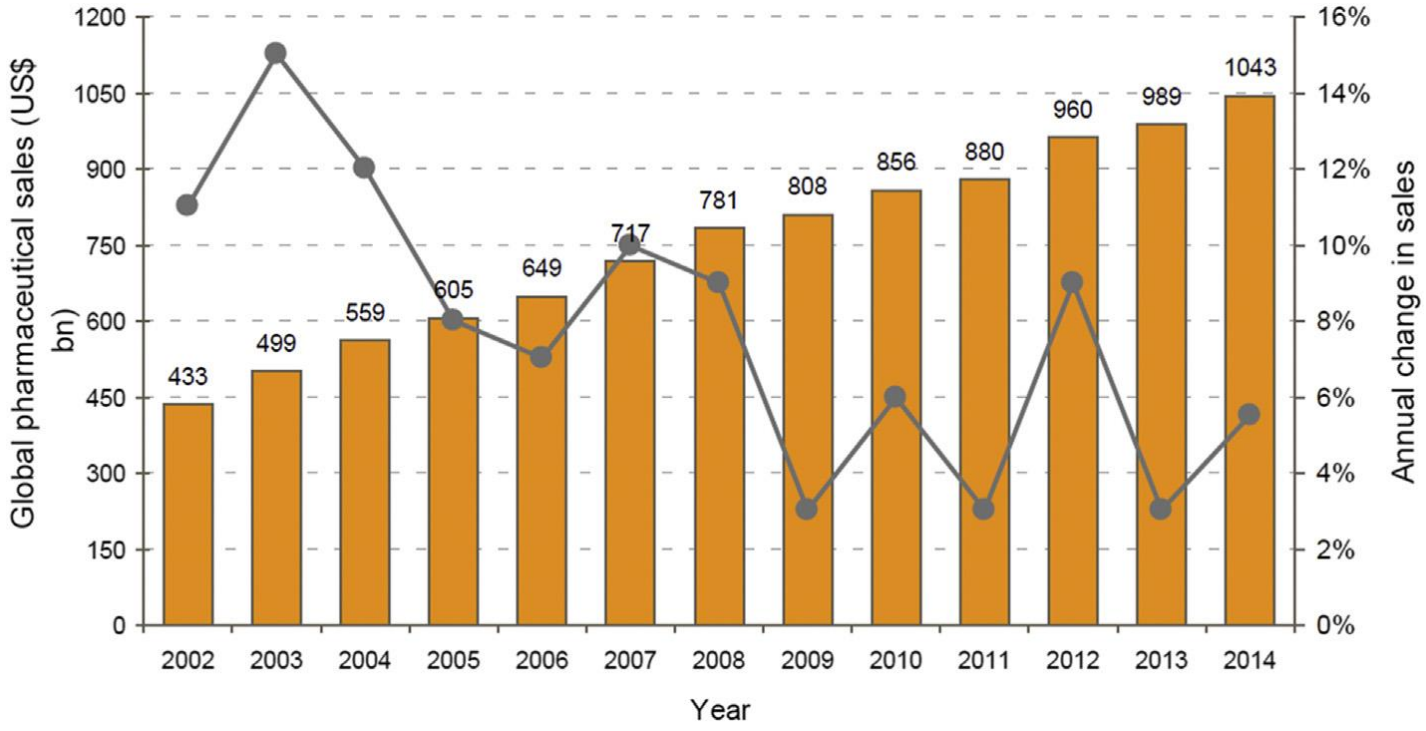
- Role of Structural Mass Spectrometry technologies for drug discovery and development

- Hydroxyl radical footprinting (HRF) workflow for mapping epitope/paratope regions and drug-protein interactions.

- Example of HRF application for mapping drug-protein interaction.

Global Pharmaceutical sales, 2002-2014

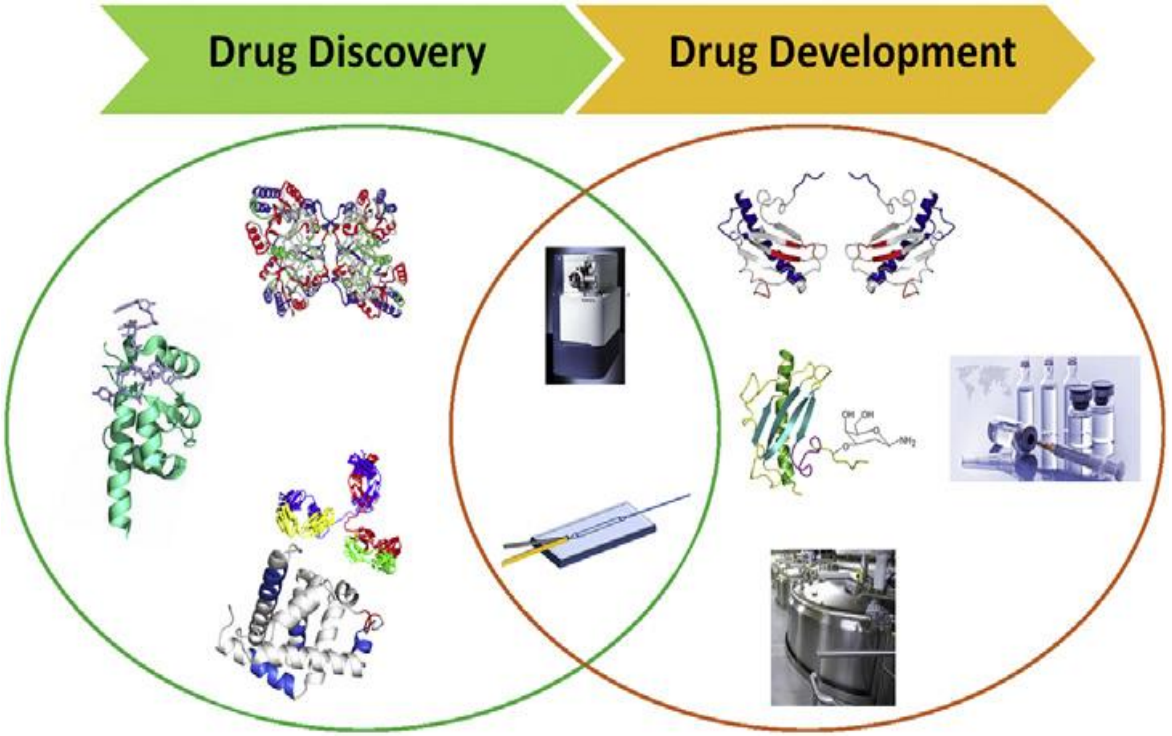
- By 2015 worldwide pharmaceutical sail reach $ 1 trillion dollars
- 12-15 years to develop and approve new drug,
- Cost for drug bringing new drug to market is $1.3 billion



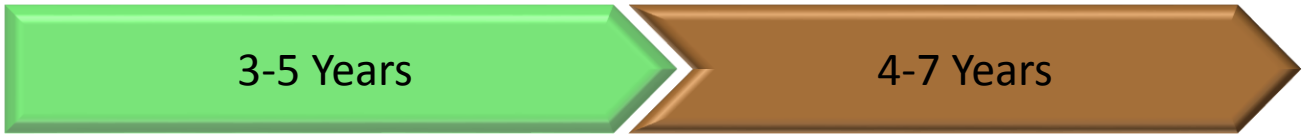
From 2015 CMR International Pharmaceutical R&D Facktbook. Produced with permission from Thomson Reuters® 2015.

Drug Discovery & Development

Drug Discovery
- Target ID
- Protein therapeutics (GPCR, mAbs)
- mAb-drug conjugates
- Protein-Ligand interaction
- Protein-Protein interaction

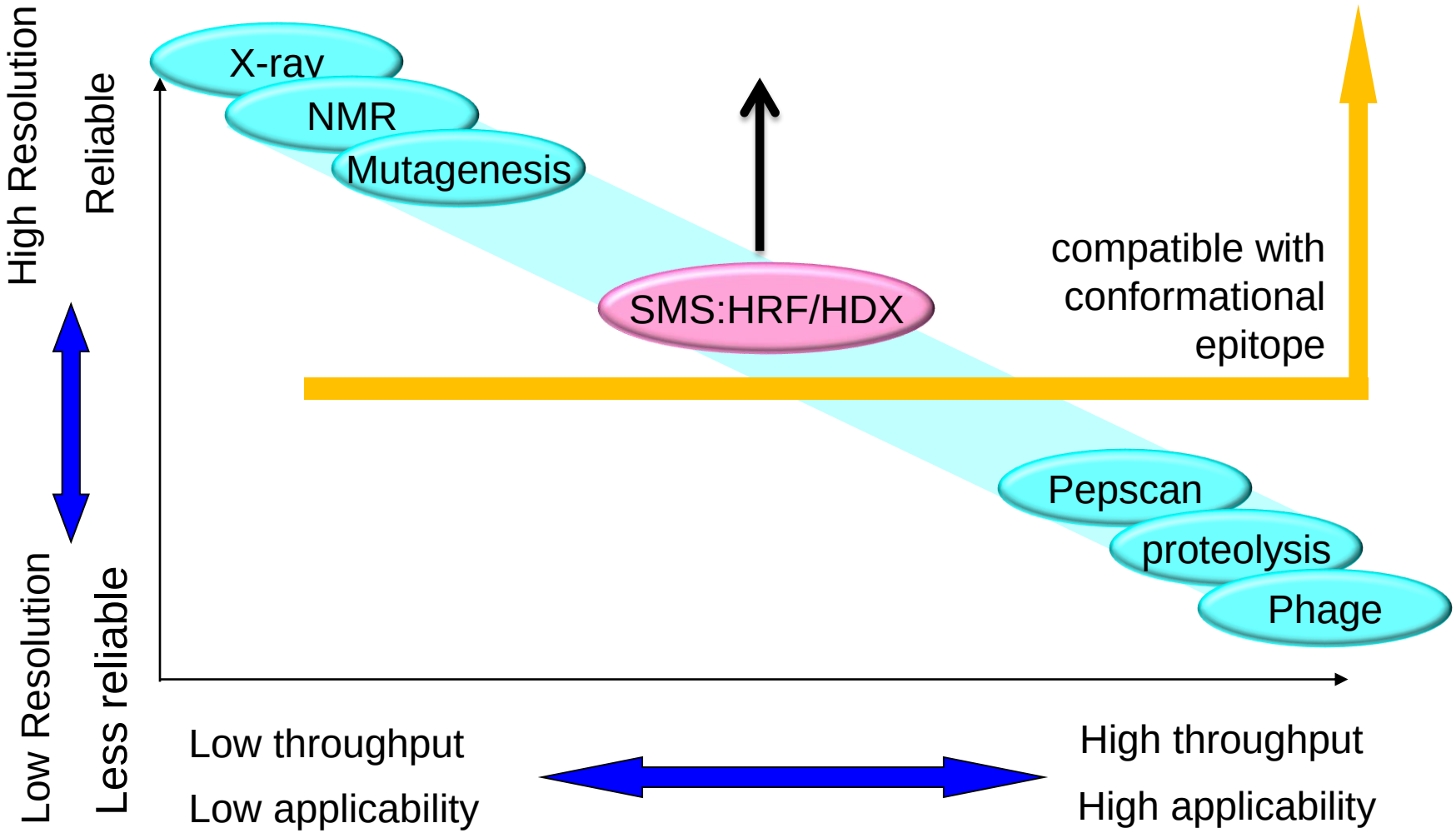


Drug Development
- Structural modifications
- Biosimilar antibodies
- Formulation
- Manufacturing



Bin Deng et al., Analytica Chemica Acta, 2016

Structure assessment/epitope mapping

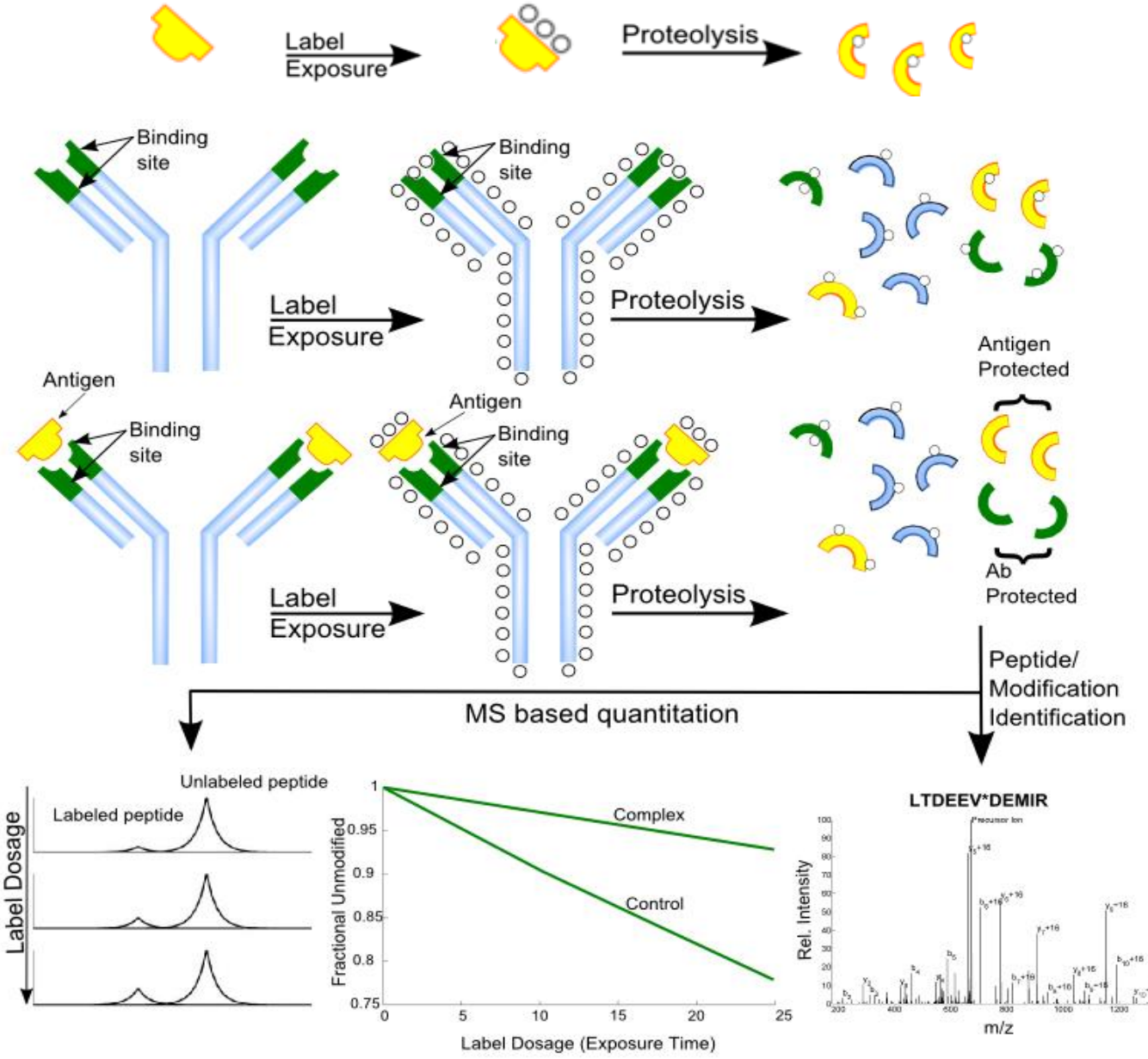


Courtesy of Yoshi Hamuro

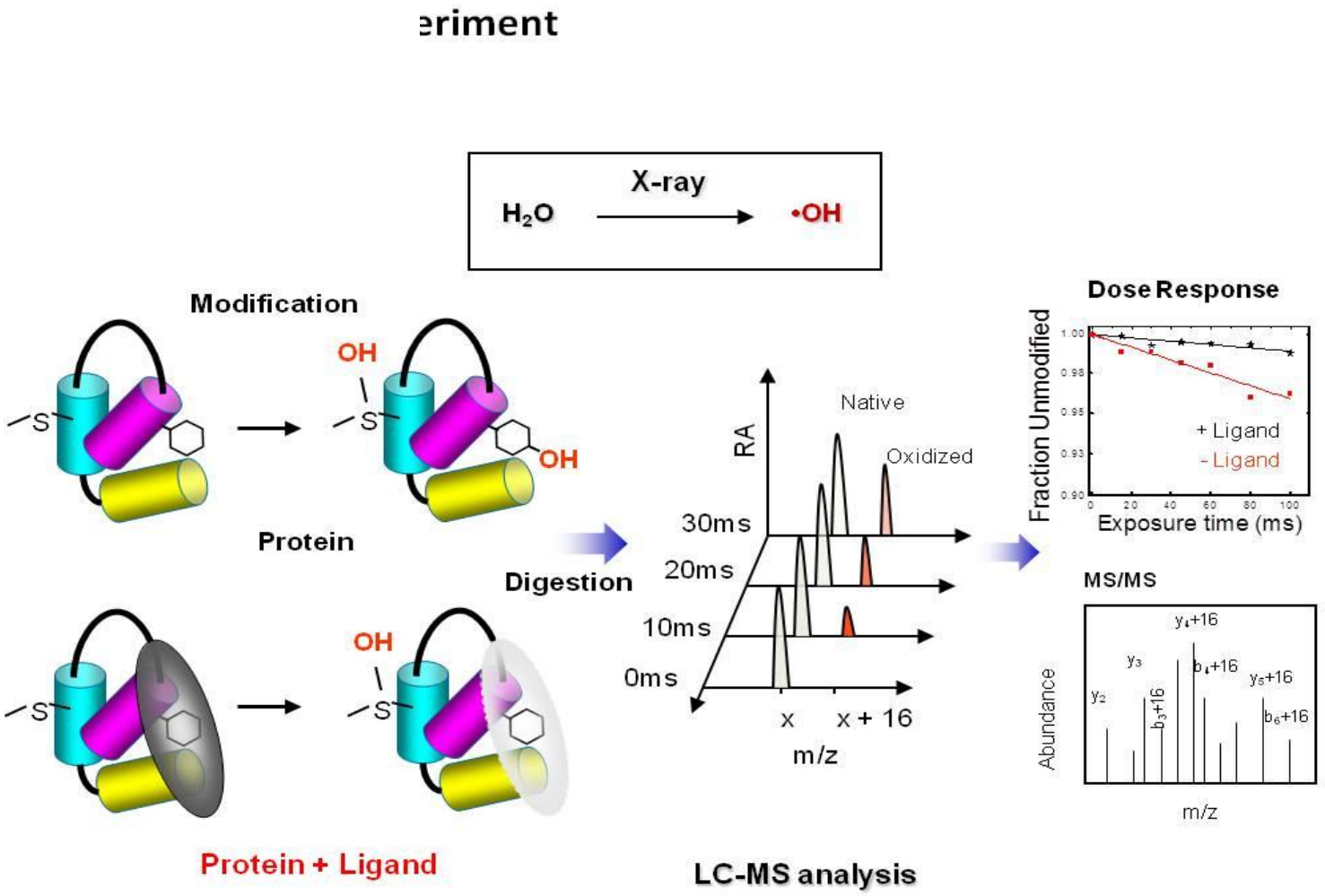
Structural Mass Spectrometry for Pharma

- Mass spectrometry technologies can rapidly map conformational epitopes. Epitope mapping is critical for establishing intellectual property protection of biologic drugs.
- Mass spectrometry technologies can efficiently assess small molecule drug-protein interactions and their mechanism of action. Understanding protein-small molecule drug interactions are critical for structure based drug development.

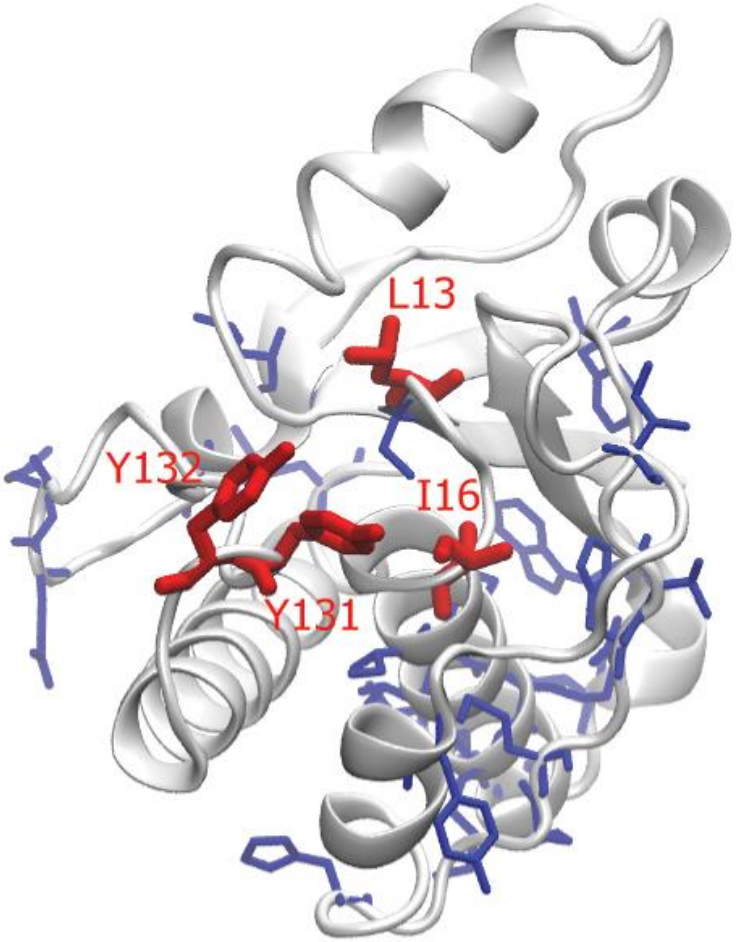
HRF workflow for Antibody-Antigen Complex Mapping



HRF workflow for Mapping drug-binding site



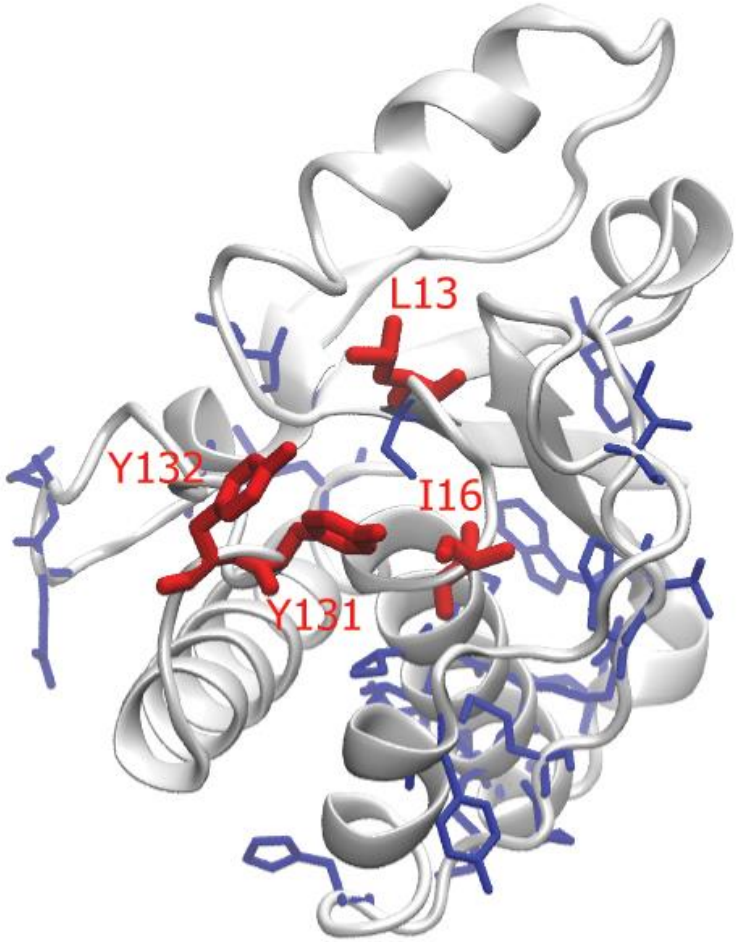
HRF results



Experimental objectives & set up:

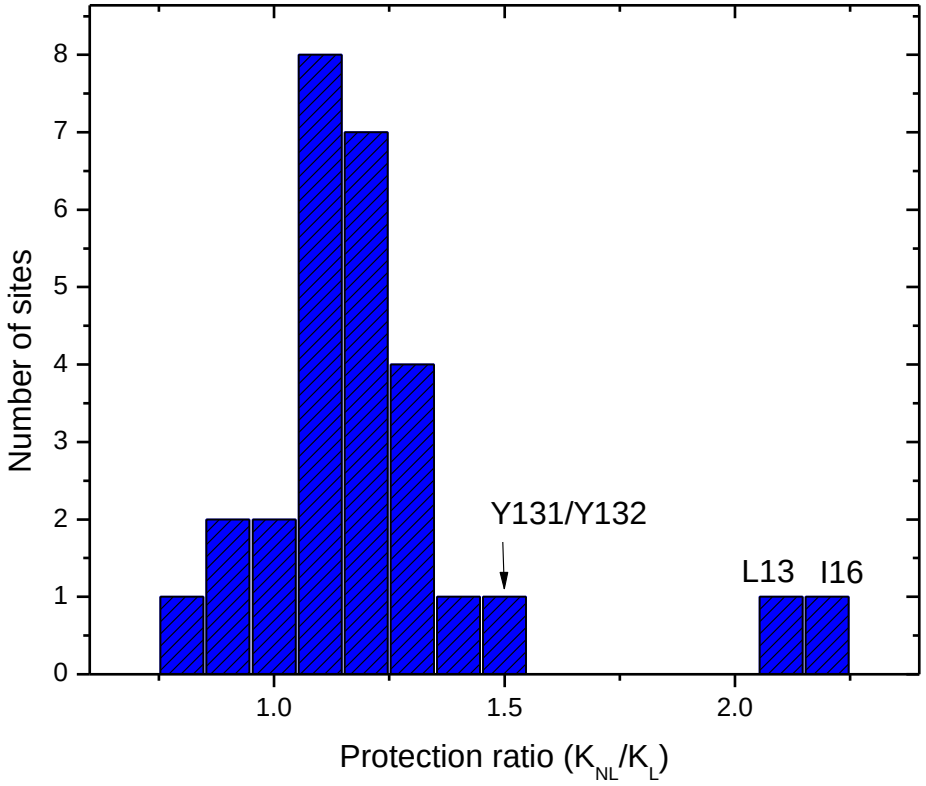
- Define a drug binding site on low molecular weight protein tyrosine phosphatase protein.
- Examine structural changes of this tyrosine phosphatase upon drug component binding.
- Protein -/+ drug was exposed for 0-800 µs.
- All samples were digested with trypsin
- LC-MS and LC-MS/MS were performed to relatively quantify the extent of oxidation for each site of modification and identify those sites, respectively

HRF results



Peptide	Modified Residues	Modification Rate, S^{-1} ($K_{control}$)	Modification Rate, S^{-1} (K_{Li})	Protection ratio, ($K_{control}/K_{Li}$)
[7-18]	C12	1.49 ± 0.24	1.39 ± 0.16	1.1
	L13	1.41 ± 0.074	0.67 ± 0.056	**2.1**
	I16	0.03 ± 0.002	0.0135 ± 0.001	**2.2**
[19-28]	F26	0.40 ± 0.024	0.33 ± 0.024	1.2
	R27	2.21 ± 0.17	1.84 ± 0.14	1.2
	K28	1.76 ± 0.11	1.59 ± 0.16	1.1
[113-123]	L115	0.048 ± 0.0035	0.042 ± 0.003	1.1
	Y119	0.14 ± 0.0087	0.097 ± 0.005	1.4
	Q122	0.34 ± 0.012	0.24 ± 0.014	1.3
	K123	0.77 ± 0.033	0.65 ± 0.019	1.2
[124-147]	Y131/Y132	3.70 ± 0.091	2.46 ± 0.024	**1.5**

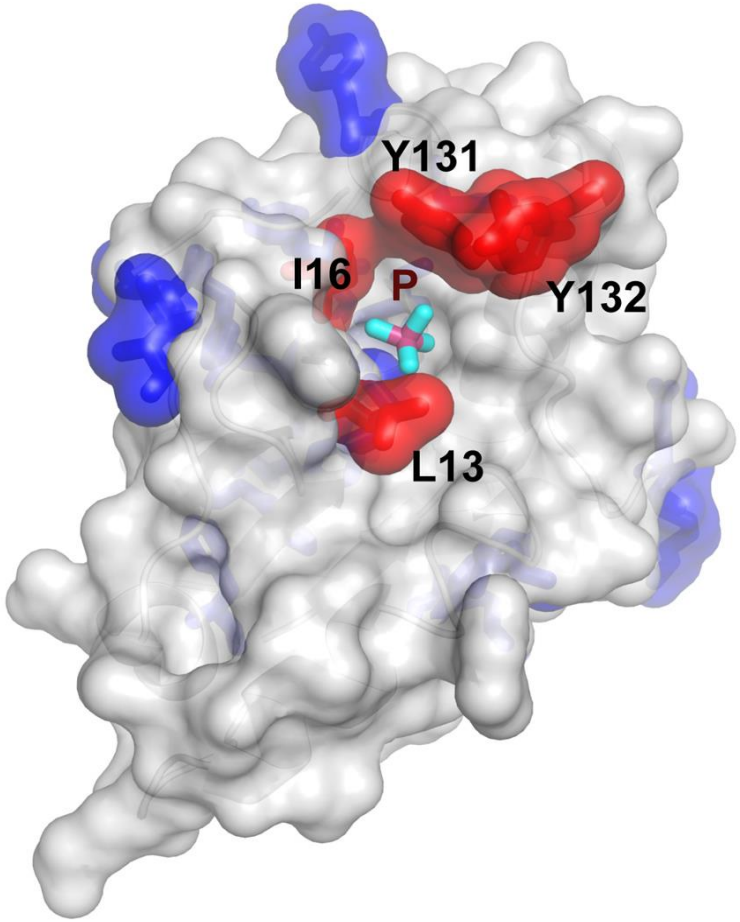
Distribution of protection ratios for the protein-Li complex



The median of the distribution is 1.1, indicating most peptides exhibit similar solvent accessibility across the protein and protein-drug complex forms. The two most protected sites L13 and I16 are marked. Other protected sites are Y131 and Y132.

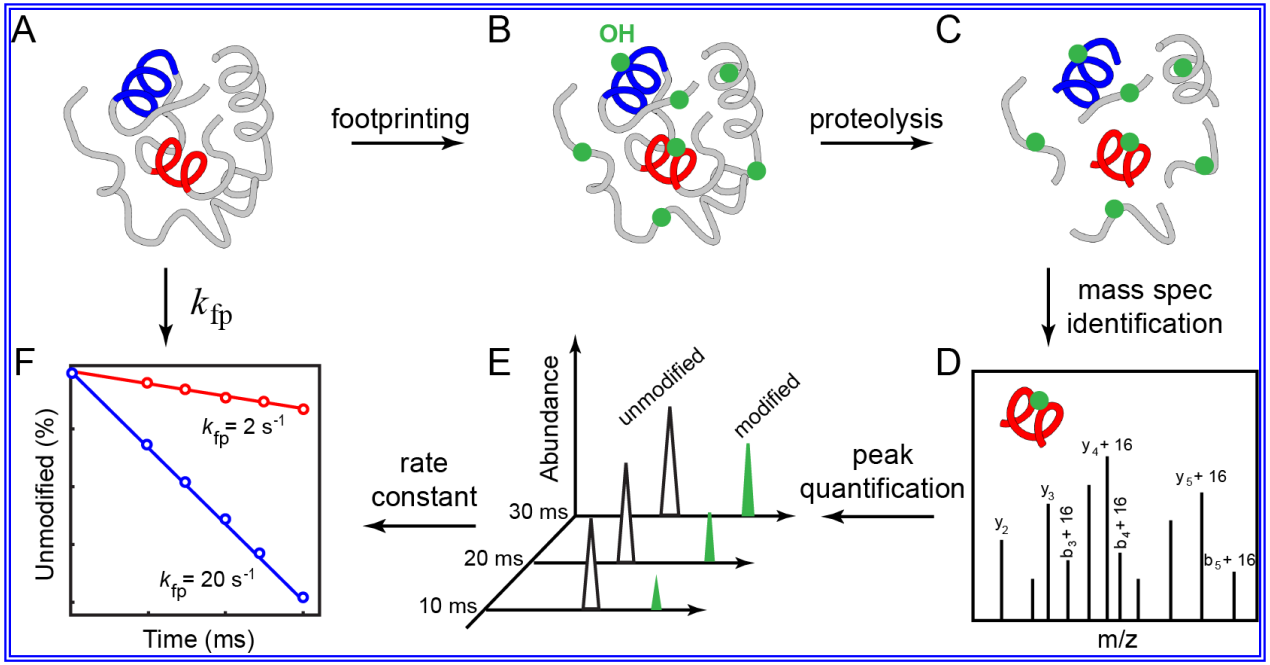
LMPTP inhibitor binding determinants

HRF



LMPTP small molecular inhibitor increases liver IR phosphorylation *in vivo, and reverses high-fat diet induced* diabetes.

Footprinting Protection Factor to Measure Protein Topography: Relative vs Absolute Assessment



$$PF = \frac{\sum_i k_{int}^i}{k_{fp}} \quad \text{OR} \quad PF = \frac{k_{int}}{k_{fp}}$$

(peptide-level) (single-residue)

OH. Intrinsic Reactivity of Amino Acids

Table 1. Rate Constants for Reaction of Amino Acids with Hydroxyl Radical and Hydrated Electrons[a]

	HO^-		e_{aq}^{-1}	
substrate	rate (M^{-1} s^{-1})	pH	rate (M^{-1} s^{-1})[b]	pH
Cys	3.5×10^{10}	7.0	1.0×10^{10}	−7
Trp	1.3×10^{10}	6.5−8.5	3.0×10^{8}	7.8
Asn	4.9×10^{7}	6.6	1.5×10^{8}	7.3
Gly	1.7×10^{7}	5.9	8.0×10^{8}	6.4

[a] http://allen.rad.nd.edu/browse compil.html. [b] Davies, M. J.; Dean, R. T. *Radical-mediated protein oxidation: from chemistry to medicine*; Oxford University Press: 1997; pp 44−45.

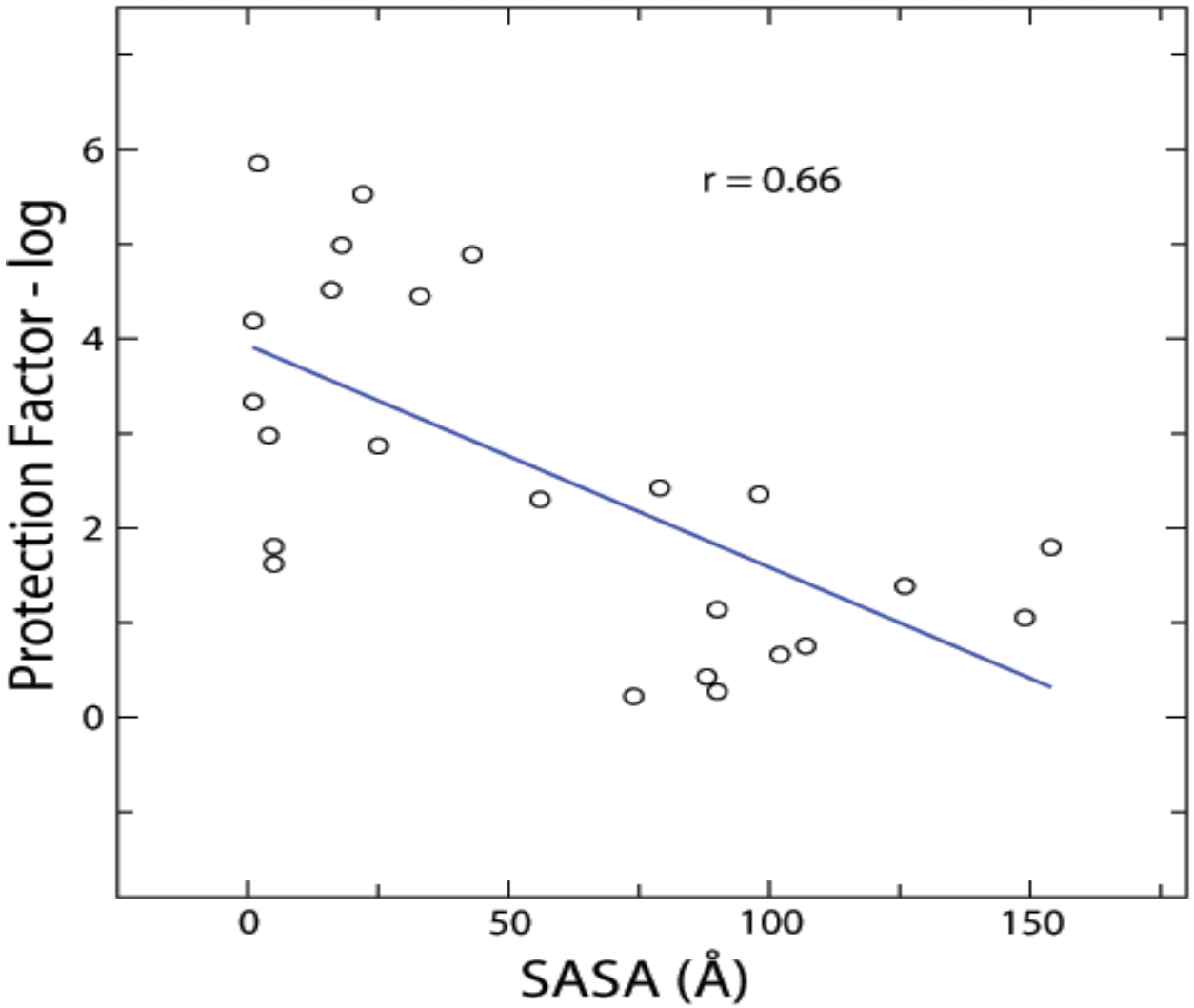
TABLE 1 Relative intrinsic reactivity (R_i) of 20 amino acids

Cys[a]	Met[b]	Trp	Tyr	Phe	His	Leu[c]	Ile[c]	Arg	Lys
29.2	20.5	17.4	12.0	11.2	9.3	4.4	4.4	2.9	2.2
Val	Thr	Ser	Pro	Glu	Gln	Asn	Asp	Ala	Gly
1.9	1.6	1.4	1.0	0.69	0.66	0.44	0.42	0.14	0.04

Xu and Chance (2005), Huang *et al,* Biophysical Journal 108 107–115 (2015)

Ri is the relative chemical reactivity for a residue-type to solution generated hydroxyl radicals, relative to an internal reference of proline reactivity

Solvent accessibility vs. reactivity (x-corr = 0.66)



Conclusion

- MS-based HRF is powerful and sensitive technique that can efficiently assess small molecule drug-protein interactions with high resolution.

- MS-based HRF is emerging as an important bioanalytical technology for protein therapeutic characterization.

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